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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 38/17 — A1

(11) International Publication Number: **WO 97/42966**

(43) International Publication Date: 20 November 1997 (20.11.97)

(21) International Application Number: PCT/US97/08016

(22) International Filing Date: 9 May 1997 (09.05.97)

(30) Priority Data:
08/644,287 10 May 1996 (10.05.96) US

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: THERAPEUTIC USES OF BPI PROTEIN PRODUCTS FOR HUMAN MENINGOCOCCEMIA

(57) Abstract

Methods and materials for the treatment of human meningococcemia are provided in which therapeutically effective amounts of BPI protein products are administered.

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THERAPEUTIC USES OF BPI PROTEIN PRODUCTS FOR HUMAN MENINGOCOCCEMIA

BACKGROUND OF THE INVENTION

The present invention relates generally to methods and materials for treating humans suffering from meningococcemia by administration of bactericidal/permeability-increasing (BPI) protein products.

Meningococcemia is an infectious disease caused by *Neisseria meningitidis* (also known as meningococcus) in which the bacteria and their products are found in the systemic circulation. Its clinical course varies from a relatively mild process to a severe, fulminant infection of sudden onset and extremely rapid progression, with the time from first fever until death spanning as little as 12 hours. The latter, dramatic form of the disease occurs in about 10% of patients infected with *N. meningitidis*. Patients may present with normal mental status and symptoms only of fever and petechiae, but may rapidly experience hemodynamic collapse, loss of the airway, and coma, along with severe coagulopathy, intravascular thrombosis, and organ failure. Alternatively, in late stages of the disease, patients may be unconscious and unresponsive at the time of presentation.

The mortality rate for acute meningococcal disease has not changed significantly over the last few decades despite technological advances in antibiotics and intensive care facilities. One retrospective study found that the mortality rate from meningococcal infection had not changed significantly over 30 years, even after adjusting for disease severity [Havens et al., *Pediatr. Infect. Dis. J.*, *8*:8-11 (1989)]. Another prospective study of meningococcal infections [Powars et al., *Clin. Infect. Dis.*, *17*:254-261 (1993)] in the years 1986 through 1991 reported that 113 patients with bacteriologically proven *N. meningitidis* infection were observed, of whom 15 (13%) died. This mortality rate of 13% had not changed appreciably from the mortality rate of 16% reported five decades earlier in a Chilean epidemic.

An "epidemic" is defined as an increased frequency of disease due to a single bacterial clone spread through a population. Although epidemics of meningococcemia are widespread in the developing world, no national epidemic has occurred in the United States since the 1940's. However, a significant increase in the endemic occurrence of meningococcemia, along with localized epidemics has occurred

in the mid-1990s. The disease continues to be seasonal, with peak incidence in the late winter and early spring. Between 60% and 90% of all cases occur in children, with the peak incidence in children under age 2.

N. meningitidis is an encapsulated gram-negative coccus, typically occurring in pairs (diplococci), which is responsible for a spectrum of severe diseases, including meningococcemia. Meningococci are divided into nine serogroups on the basis of their capsular polysaccharides, with serogroups A, B, C, Y, and W135 accounting for the majority of clinical disease. These serogroups are further subdivided into antigenically distinct serotypes on the basis of expression of outer membrane proteins. Specific clones within each serogroup can be further delineated by protein electrophoretic patterns. The outer membrane of meningococci also contains a form of lipopolyaccharide (LPS), i.e., "lipooligosaccharide" (LOS), which is a common component of the outer membrane of gram-negative bacteria.

The meningococcus is known to colonize the nasopharynx of 5-15% of individuals; however, only a small fraction of those colonized will experience invasive disease. The transition from colonization to invasive disease is multifactorial and incompletely understood. The presence of viral upper-respiratory infections, which also peak during the late winter and spring, may damage the nasopharyngeal epithelium and permit bacterial translocation across an altered barrier. In children under 2 years of age, inadequate development of antibodies directed against the meningococcal polysaccharide capsule is thought to account for the high attack rate in this population.

The spectrum of disease caused by the meningococcus includes meningitis, arthritis, pericarditis, endocarditis, conjunctivitis, endophthalmitis, respiratory tract infections, abdominal and pelvic infections, urethritis, and a chronic bacteremic syndrome. The predominant clinical syndromes requiring pediatric intensive care unit (PICU) admission are meningitis and meningococcemia (with or without meningitis). The clinical presentation depends on the compartment of the body in which the infection and its inflammatory sequelae are primarily localized.

In contrast to meningococcemia, meningitis is a disease in which the bacteria are localized to the meningeal compartment, with signs consistent with meningeal irritation. Clinically, meningococcal meningitis is dramatically different

from meningococcemia, however, it may be indistinguishable from other forms of meningitis, and only differentiated by culture or immunologic assays. Systemic hemodynamic signs, severe coagulopathy and intravascular thrombosis are notably absent. If properly treated, mortality is rare and neurologic sequelae, including sensineural hearing loss, is uncommon. The approach to diagnosis and treatment of meningococcal meningitis is the same as with other forms of bacterial meningitis.

If patients are examined early in the course of their disease when only petechiae and mild constitutional symptoms are evident, the diagnosis of meningococcemia may be complicated by the number of diseases which present with fever and petechiae in children, including, for example, infections by enterovirus, rotavirus, respiratory syncytial virus, *Haemophilus influenzae*, or *Streptococcus pneumoniae*; streptococcal pharyngitis; Rocky Mountain spotted fever, Henoch-Schoenlein purpura; or malignancy. However, since the outcome of meningococcal disease is highly dependent on rapid diagnosis and institution of antibiotics, the suspicion of meningococcemia must be aggressively pursued and treatment instituted, particularly since *H. influenza* meningitis has markedly decreased in the United States due to use of the vaccine against the bacteria.

Like other gram negative infections, the pathogenesis of severe meningococcemia is initiated by the endotoxin on, associated with or released from the bacteria. This bacterial endotoxin activates the pro-inflammatory cytokine cascade. In severe meningococcemia, the levels of bacterial endotoxin detected in the circulation by the LAL assay have been documented to be as much as 50-100 fold greater than levels documented in other gram negative infections. The complement cascade is also activated by bacteria and their endotoxin in the systemic circulation, producing anaphylotoxins which may mediate early hypotension and capillary leak.

In studies thus far, plasma levels of endotoxin [Brandtzaeg et al., *J. Infec. Dis.*, *159*:195-204 (1989)], TNF [Van Deuren et al., *J. Infect. Dis.*, *172*:433-439 (1995)], IL-6 [Van Deuren et al., *supra*], and fibrinogen, as well as prothrombin time (PTT) [McManus et al., *Critical Care Med.*, *21*:706-711 (1993)] in meningococcemia patients have been correlated with the severity and outcome of disease, although the correlation is imprecise. It has been suggested that combining ranked values for endotoxin, TNF, IL-1 and IL-6 can achieve a score that accurately

reflects patient outcome [Bone, *Critical Care Med.*, *22*:S8-S11 (1994)].

Severe coagulopathy and intravascular thrombosis may be rapidly progressive and lead to ischemic injury of extremities and vital organs in meningococcemia patients. Respiratory failure, renal failure, adrenal failure and coma may develop. Petechiae and purpura may be extensive and become confluent, in which case the term "purpura fulminans" has been applied. In meningococcemic patients with severe disease, significant reductions in the coagulation inhibitors antithrombin III, activated protein C, and protein S have also been documented. These reductions may reflect a relative imbalance of anti-coagulant factors compared to pro-coagulants, but may also reflect the general consumption of all classes of factors. Quantitative deficiencies may also reflect hemodilution and capillary leak of proteins.

Severe cardiac dysfunction is often present on admission, or may develop within the first 24 hours. Ejection fractions of 20% or less are frequent. Cardiac dysfunction may be secondary to a number of factors, including: 1) myocarditis, which is present to varying degrees in a majority of autopsy specimens; 2) myocardial depressant substances; 3) intravascular thrombosis and subsequent myocardial ischemia; 4) myocardial interstitial edema, resulting in a non-compliant ventricle; 5) hypoxic myocardial injury; and 6) metabolic abnormalities.

Hypotension and circulatory insufficiency are multifactorial, with significant contributions from intravascular volume depletion, capillary leak, profound vasodilation (secondary to anaphylotoxins, nitric oxide, histamine, and other mediators), and depressed myocardial performance. Organ damage secondary to hypotension, intravascular thrombosis, and direct inflammatory damage may be evident at presentation.

Fulminant disease may be associated with adrenal hemorrhage, adrenal cortical necrosis, and rapid demise (Waterhouse Friederickson Syndrome). Even extensive adrenal hemorrhages, however, do not necessarily denote adrenal insufficiency, since normal or even elevated systemic cortisol levels have been documented in such patients. In a minority of patients with rapidly progressive disease, adrenal hemorrhages are associated with serum cortisol levels which are normal or subnormal (in a setting where elevated levels are expected). Other

metabolic derangements such as metabolic acidosis, hypoglycemia, hypocalcemia, and hypomagnesemia are also frequently present.

Patients with severe disease are at highest risk of mortality. If they survive, they often experience severe morbidities, including extensive tissue and bone destruction that requires debridement and/or amputation followed by skin grafting procedures. In one study [Powars et al., *supra*], among the 28 patients with purpura fulminans, the hallmark of severe meningococcemia, 14 patients (50%) died. Of the 14 surviving patients who had purpura fulminans, 10 suffered soft tissue gangrene with deforming autoamputation. In another report [Genoff et al., *Plastic Reconstructive Surg., 89*:878-881 (1992)], six patients with meningococcemia and purpura fulminans were followed, of whom four patients required severe amputations (wrist or above for the upper limbs, or ankle or above for the lower limbs). Genoff et al. note that even after the life-threatening acute phase of the disease has passed, complications continue and require revisions to a higher level of amputation and multiple grafting procedures. Sheridan et al., *Burns, 22*:53-56 (1996), confirms that meningococcemia with purpura fulminans has a reported mortality rate of 50%, with high rates of major amputations in survivors. In their experience, surviving patients are often left with full thickness wounds involving the skin, subcutaneous tissue and often underlying muscle and bone; half of the surviving patients require major amputations.

Patients with meningococcal disease may also develop neurologic sequelae, including electroencephalogram (EEG) abnormalities, computerized tomography (CT) scan abnormalities, hearing impairment and neuropsychological testing deficits. In one study, 99 consecutive children and adult patients with acute, bacteriologically confirmed meningococcal disease were followed and tested for neurologic sequelae one year after their illness. [Naess et al., *Acta Neurol. Scand., 89*:139-142 (1994).] In the category of patients suffering from meningococcemia with hypotension and/or ecchymoses, but without signs of meningitis, neurologic sequelae were observed in 5 of the 12 patients. In the category of patients suffering from meningococcemia with hypotension and/or ecchymoses, and with signs of meningitis, neurologic sequelae were observed in 7 of 13 patients.

Clinical outcome can be reasonably predicted by scoring of risk factors

originally identified in large cohorts of meningococcemia patients. In 1966, Stiehm and Damrosh, *J. Pediatrics, 68*:457-467 (1966), reviewed 63 cases of meningococcal infection and identified clinical features associated with poor outcome. Poor prognostic factors included: onset of petechiae within 12 hours prior to admission, absence of meningitis (cerebrospinal fluid (CSF) WBC <20), shock (systolic blood pressure <70), normal or low white blood count (WBC $<10,000$), and normal or low erythrocyte sedimentation rate (<10 mm/hr). The presence of 3 or more of these criteria was associated with poor outcome. Niklasson et al., *Scand. J. Infect. Dis., 3*:17-25 (1971), substantiated these risk factors in 1971, and added temperature $>40^{\circ}C$ and thrombocytopenia to the list of poor prognostic signs. The specific predictive abilities of the Stiehm and Damrosh criteria and the Niklasson criteria have been challenged in a series from McManus [McManus et al., *supra*] in 1993. In this series, mortality was significantly less than predicted by earlier criteria and was more likely related to the presence or absence of coagulopathy.

The most widely used meningococcal sepsis scoring system was published in 1987 by Sinclair et al., *Lancet, 2*:38 (1987), and has become known as the Glasgow Meningococcal Septicemia Prognostic Score (Glasgow score). Its utility stems from its reliance on bedside clinical indicators, which facilitates triage in the field or during transport. Points are given on a rated scale for seven parameters as follows: (1) BP $<$ 75 mm Hg systolic, age $<$ 4 years or BP $<$ 85 mm Hg systolic, age $>$ 4 years (3 points); (2) skin/rectal temperature difference $>3^{\circ}C$ (3 points); (3) modified coma scale $<$ 8, or deterioration of 3 or more points in 1 hour (3 points); (4) deterioration in hour before scoring (2 points); (5) absence of meningism (2 points); (6) extending purpura or widespread ecchymoses (1 point); and (7) base deficit (capillary or arterial) $>$ 8 (1 point). The maximum Glasgow score is therefore 15 points.

Since meningococcemia is frequently characterized by rapid and fulminant deterioration, vigilant monitoring is mandated. The great majority of patients should be admitted directly to the intensive care unit, where invasive monitoring can be instituted, and supportive therapy provided. Specific additions to monitoring and laboratory evaluation may include obtaining samples from CSF, blood cultures, skin lesions and throat swabs. However, CSF should be obtained only if

the patient's clinical condition is stable enough to tolerate the procedure. Blood cultures should be obtained, but are positive in only 50% of untreated patients. Bacteria can also be detected in up to 70% of cases by Gram stain and culture of aspirated (or biopsied) hemorrhagic skin lesions. Examination of skin lesions is especially important for cases in which antibiotics have been administered prior to obtaining blood cultures. Throat swabs, if carefully obtained and rapidly plated, may also yield meningococci and support a presumptive diagnosis of meningococcemia. Alternatively, CSF may be obtained for detection of meningococcal antigens. If an organism is obtained, it should be serotyped and forwarded to a reference laboratory for additional subtyping. Epidemic control through immunization can only occur if the specific organisms responsible for disease are identified. In the unusual circumstance in which blood cultures cannot be obtained, antibiotics should still be administered without delay; microbiologic investigation can be accomplished at a later time by alternate methods.

Management of children with meningococcemia relies on intensive, aggressive monitoring and therapy. In particular, early protection of the airway, aggressive volume replacement, and appropriate institution of vasoactive agents, e.g., epinephrine, dopamine and dobutamine, are critical to restore tissue perfusion and oxygen delivery. A few specific issues in the treatment of meningococcemia, including treatment with antibiotics, steroids, fresh frozen plasma (FFP) replacement, heparin, and several new agents are briefly highlighted below.

An ongoing debate continues concerning whether antibiotics should be administered as soon as the diagnosis is suspected or after a period of stabilization. Although not resolved by randomized trials, the preponderance of evidence suggests that antibiotics should be administered immediately, while other supportive therapies are being instituted. Speculations regarding a post-antibiotic release of bacterial endotoxin in meningococcemia have not been substantiated by human data. Serial quantitation of bacterial endotoxin levels in plasma samples from humans with meningococcemia have failed to demonstrate a post-antibiotic surge in plasma endotoxin levels.

Initial therapy of suspected cases currently is typically recommended to be a third generation cephalosporin (e.g. Ceftriaxone) until other causes of severe

infectious purpura with shock have been ruled out (*H. influenza, S. pneumoniae*, other gram negative bacteria). Therapy can then be switched to parenteral penicillin or ampicillin.

To date, there are currently no randomized, placebo controlled data to support the routine use of corticosteroids in patients with meningococcemia. However, data have demonstrated that a minority of patients with severe disease and adrenal hemorrhage exhibit normal or subnormal levels of plasma cortisol (in a situation during which elevated levels are expected). Although the lack of data precludes an affirmative or negative recommendation, the physician should consider administering adrenal replacement steroids (hydrocortisone 1-2 mg/kg i.v.) in a clinical situation of rapidly progressive shock that is unresponsive to fluids and inotropes.

There have also been to date no randomized, placebo controlled data to determine whether, or to what degree, biochemical coagulopathy should be treated with FFP. Although correction of biochemical abnormalities may appear logical, administration of FFP has been viewed by many as "fueling the fire" of coagulopathy. In a case-control trial of 336 patients in Norway, treatment with plasma or blood products (as opposed to albumin or plasma substitutes) was *independently* associated with poorer outcome. A surge in plasma endotoxin was also documented in a C6 deficient human following FFP administration during treatment for meningococcemia. These data suggest that administration of FFP may be harmful in some situations and therefore should be done carefully and only when there are compelling clinical indications.

Although small retrospective reports advocate the use of heparin as a treatment for purpura fulminans, the preponderance of data (small randomized trials and large case-control studies) do not indicate a beneficial effect of heparin therapy. There is currently no evidence to support the routine use of heparin in the treatment of meningococcemia. A large scale, double-blind, placebo-controlled Phase III trial of a monoclonal anti-lipid A antibody (HA-1A) in meningococcemia has been conducted in Europe. No results have been published to date.

In addition, a number of other biological agents are candidates for treatment of severe coagulopathy and intravascular thrombosis. These agents include:

antithrombin III, protein C, and tissue factor pathway inhibitor. Anecdotal experiences with protein C and antithrombin III have already been published pending definitive trials. Other clinical interventions have been reported but have not been systematically tested, including: plasma and whole blood exchange, leukaplasmapheresis, continuous caudal blockade to relieve lower extremity ischemia, and topical application of nitroglycerin to vasodilate the peripheral vascular bed.

BPI is a protein isolated from the granules of mammalian polymorphonuclear leukocytes (PMNs or neutrophils), which are blood cells essential in the defense against invading microorganisms. Human BPI protein has been isolated from PMNs by acid extraction combined with either ion exchange chromatography [Elsbach, *J. Biol. Chem.*, *254*:11000 (1979)] or *E. coli* affinity chromatography [Weiss, et al., *Blood, 69*:652 (1987)]. BPI obtained in such a manner is referred to herein as natural BPI and has been shown to have potent bactericidal activity against a broad spectrum of gram-negative bacteria. The molecular weight of human BPI is approximately 55,000 daltons (55 kD). The amino acid sequence of the entire human BPI protein and the nucleic acid sequence of DNA encoding the protein have been reported in Figure 1 of Gray et al., *J. Biol. Chem.*, *264*:9505 (1989), incorporated herein by reference. The Gray et al. amino acid sequence is set out in SEQ ID NO: 1 hereto. U.S. Patent No. 5,198,541 discloses recombinant genes encoding and methods for expression of BPI proteins, including BPI holoprotein and fragments of BPI.

BPI is a strongly cationic protein. The N-terminal half of BPI accounts for the high net positive charge; the C-terminal half of the molecule has a net charge of +3. [Elsbach and Weiss (1981), *supra.*] A proteolytic N-terminal fragment of BPI having a molecular weight of about 25 kD possesses essentially all the anti-bacterial efficacy of the naturally-derived 55 kD human BPI holoprotein. [Ooi et al., *J. Bio. Chem.*, *262*: 14891-14894 (1987)]. In contrast to the N-terminal portion, the C-terminal region of the isolated human BPI protein displays only slightly detectable anti-bacterial activity against gram-negative organisms. [Ooi et al., *J. Exp. Med.*, *174*:649 (1991).] An N-terminal BPI fragment of approximately 23 kD, referred to as "$rBPI_{23}$," has been produced by recombinant means and also retains anti-bacterial activity against gram-negative organisms. Gazzano-Santoro et al., *Infect. Immun.*

60:4754-4761 (1992).

The bactericidal effect of BPI has been reported to be highly specific to gram-negative species, e.g., in Elsbach and Weiss, *Inflammation: Basic Principles and Clinical Correlates*, eds. Gallin et al., Chapter 30, Raven Press, Ltd. (1992). The precise mechanism by which BPI kills gram-negative bacteria is not yet completely elucidated, but it is believed that BPI must first bind to the surface of the bacteria through electrostatic and hydrophobic interactions between the cationic BPI protein and negatively charged sites on LPS. In susceptible gram-negative bacteria, BPI binding is thought to disrupt LPS structure, leading to activation of bacterial enzymes that degrade phospholipids and peptidoglycans, altering the permeability of the cell's outer membrane, and initiating events that ultimately lead to cell death. [Elsbach and Weiss (1992), *supra*]. LPS has been referred to as "endotoxin" because of the potent inflammatory response that it stimulates, i.e., the release of mediators by host inflammatory cells which may ultimately result in irreversible endotoxic shock. BPI binds to lipid A, reported to be the most toxic and most biologically active component of LPS.

BPI has never been used previously for the treatment of subjects infected with *N. meningitidis*, including subjects suffering from meningococcemia. In co-owned, co-pending U.S. Application Serial Nos. 08/378,228, filed January 24, 1995, 08/291,112, filed August 16, 1994, and 08/188,221, filed January 24, 1994, incorporated herein by reference, the administration of BPI protein product to humans with endotoxin in circulation was described. [See also, von der Möhlen et al., *J. Infect. Dis. 172*:144-151 (1995); von der Möhlen et al., *Blood 85*:3437-3443 (1995); de Winter et al., *J. Inflam. 45*:193-206 (1995)]. Thornton et al., *FASEB J.*, 8(4):A137, 1994, report that BPI inhibited the release of TNF *in vitro* by human inflammatory cells in response to LOS derived from two *Neisseria* species, *N. meningitidis* and *N. gonorrhea*; and the report in International Application Publication No. WO 94/25476 published November 10, 1994, of methods of treating endotoxin-related disorders, including Gram-negative meningitis.

In spite of treatment with antibiotics and state-of-the-art medical intensive care therapy, the mortality and morbidities associated with human meningococcemia remain significant and unresolved by current therapies. New

therapeutic methods are needed that could reduce or ameliorate the adverse events and improve the clinical outcome of human meningococcemia, including, for example, reducing mortality, amputations, grafting procedures, permanent neurologic impairment and improving pediatric outcome scores.

SUMMARY OF THE INVENTION

The present invention provides novel methods for treatment of humans with meningococcemia involving the administration of BPI protein products to provide clinically verifiable alleviation of the adverse effects of, or complications associated with, this human disease, including mortality and morbidities.

According to the invention, BPI protein products such as $rBPI_{21}$ are administered to humans suffering from meningococcemia in amounts sufficient to prevent mortality and/or to reduce the number or severity of morbidities, including but not limited to amputations, grafting procedures and/or permanent neurologic impairment. Also contemplated is use of a BPI protein product in the preparation of a medicament for the treatment of meningococcemia in humans.

Numerous additional aspects and advantages of the invention will become apparent to those skilled in the art upon consideration of the following detailed description of the invention which describes presently preferred embodiments thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1, 3 and 5 depict plasma levels of endotoxin, TNF and IL-6, respectively, over time for the initial ten patients enrolled in the BPI study.

Figures 2, 4 and 6 depict the plasma endotoxin, TNF and IL-6 levels displayed in Figures 1, 3 and 5, respectively, offset for each patient by the time between initiation of antibiotic treatment and initiation of BPI protein product therapy.

DETAILED DESCRIPTION

Human meningococcemia is an increasingly prevalent, life-threatening, debilitating disease for which conventional antibiotics and intensive care are inadequate. In particular, significant mortality and severe morbidities have remained

in spite of state-of-the-art medical intensive care. It has now been unexpectedly found that the administration of BPI protein products to humans with meningococcemia has effectively decreased mortality and reduced the number and severity of morbidities, including amputations, debridement of dead tissue followed by extensive grafting procedures, and/or permanent neurologic impairment resulting in significant and long-term impairment of neurologic function (e.g., cerebrovascular accidents, cerebral atrophy, or seizures requiring medication). These unexpected effects on the mortality and morbidities associated with and resulting from meningococcemia demonstrate that BPI protein products have effectively interfered with or blocked a number of the multiple poorly-understood pathophysiologic processes that have led to poor outcomes in this human disease.

BPI protein products are expected to provide other beneficial effects for meningococcemia patients, such as reduced number of episodes of hypotension or cardiac arrhythmia or arrest, reduced length of time on ventilatory support and inotropic (vasoactive) therapy, reduced duration and severity of associated coagulopathy, reduced stay in the ICU, and reduced incidence of complications such as respiratory failure, renal failure, coma, adrenal cortical necrosis, pericarditis, endocarditis, cardiomyopathy, endophthalmitis, and arthritis.

BPI protein products have been demonstrated to have a bactericidal effect *in vitro* against serogroups A, B, C and W135 of the gram-negative bacteria, *Neisseria meningitidis*, that causes meningococcemia. BPI protein products may exert their effect in human meningococcemia through such direct bactericidal action, or through enhancing the effectiveness of antibiotic therapy as described in U.S. Patent No. 5,523,288, which is incorporated herein by reference. BPI protein products may also exert their effect in human meningococcemia through neutralizing LOS endotoxin that has been released from or remains in association with the bacteria and bacterial fragments. The effects of BPI protein products in humans with endotoxin in circulation, including effects on TNF, IL-6 and endotoxin is described in co-owned, co-pending U.S. Application Serial No. 08/378,228, filed January 24, 1995, which in turn is a continuation-in-part application of U.S. Serial No. 08/291,112, filed August 16, 1994, which in turn is a continuation-in-part application of U.S. Serial No. 08/188,221, filed January 24, 1994, all of which are incorporated herein by

reference. BPI protein products exhibit both anticoagulant and fibrinolytic effects, as described in co-owned, co-pending U.S. Application Serial No. 08/644,290 filed concurrently herewith, which is incorporated herein by reference. BPI protein products may act on other pathologic processes that accompany meningococcemia including, for example, coagulopathies.

Therapeutic compositions comprising BPI protein product may be administered systemically or topically. Systemic routes of administration include oral, intravenous, intramuscular or subcutaneous injection (including into a depot for long-term release), intraocular and retrobulbar, intrathecal, intraperitoneal (e.g. by intraperitoneal lavage), intrapulmonary using aerosolized or nebulized drug, or transdermal. The preferred route is intravenous administration. When given parenterally, BPI protein product compositions are generally injected in doses ranging from 1 μg/kg to 100 mg/kg per day, preferably at doses ranging from 0.1 mg/kg to 20 mg/kg per day, more preferably at doses ranging from 1 to 20 mg/kg/day and most preferably at doses ranging from 2 to 10 mg/kg/day. The treatment may continue by continuous infusion or intermittent injection or infusion, at the same, reduced or increased dose per day for, *e.g.*, 1 to 3 days, and additionally as determined by the treating physician. BPI protein products are preferably administered intravenously by an initial bolus followed by a continuous infusion. The preferred regimen is a 1 to 20 mg/kg intravenous bolus of BPI protein product followed by intravenous infusion at a dose of 1 to 20 mg/kg/day, continuing for up to one week. The most preferred dosing regimen is a 2 to 10 mg/kg initial bolus followed by intravenous infusion at a dose of 2 to 10 mg/kg/day, continuing for up to 72 hours. Topical routes include administration in the form of salves, ophthalmic drops, ear drops, irrigation fluids (for, e.g., irrigation of wounds) or medicated shampoos. For example, for topical administration in drop form, about 10 to 200 μL of a BPI protein product composition may be applied one or more times per day as determined by the treating physician. Those skilled in the art can readily optimize effective dosages and administration regimens for therapeutic compositions comprising BPI protein product, as determined by good medical practice and the clinical condition of the individual patient.

As used herein, "BPI protein product" includes naturally and

recombinantly produced BPI protein; natural, synthetic, and recombinant biologically active polypeptide fragments of BPI protein; biologically active polypeptide variants of BPI protein or fragments thereof, including hybrid fusion proteins and dimers; biologically active polypeptide analogs of BPI protein or fragments or variants thereof, including cysteine-substituted analogs; and BPI-derived peptides. The BPI protein products administered according to this invention may be generated and/or isolated by any means known in the art. U.S. Patent No. 5,198,541, the disclosure of which is incorporated herein by reference, discloses recombinant genes encoding and methods for expression of BPI proteins including recombinant BPI holoprotein, referred to as $rBPI_{50}$ (or rBPI) and recombinant fragments of BPI. Co-owned, copending U.S. Patent Application Ser. No. 07/885,501 and a continuation-in-part thereof, U.S. Patent Application Ser. No. 08/072,063 filed May 19, 1993 and corresponding PCT Application No. 93/04752 filed May 19, 1993, which are all incorporated herein by reference, disclose novel methods for the purification of recombinant BPI protein products expressed in and secreted from genetically transformed mammalian host cells in culture and discloses how one may produce large quantities of recombinant BPI products suitable for incorporation into stable, homogeneous pharmaceutical preparations.

Biologically active fragments of BPI (BPI fragments) include biologically active molecules that have the same or similar amino acid sequence as a natural human BPI holoprotein, except that the fragment molecule lacks amino-terminal amino acids, internal amino acids, and/or carboxy-terminal amino acids of the holoprotein. Nonlimiting examples of such fragments include a N-terminal fragment of natural human BPI of approximately 25 kD, described in Ooi et al., *J. Exp. Med.*, *174*:649 (1991), and the recombinant expression product of DNA encoding N-terminal amino acids from 1 to about 193 or 199 of natural human BPI, described in Gazzano-Santoro et al., *Infect. Immun.* *60*:4754-4761 (1992), and referred to as $rBPI_{23}$. In that publication, an expression vector was used as a source of DNA encoding a recombinant expression product ($rBPI_{23}$) having the 31-residue signal sequence and the first 199 amino acids of the N-terminus of the mature human BPI, as set out in Figure 1 of Gray et al., *supra*, except that valine at position 151 is specified by GTG rather than GTC and residue 185 is glutamic acid (specified by

GAG) rather than lysine (specified by AAG). Recombinant holoprotein ($rBPI_{50}$) has also been produced having the sequence (SEQ ID NOS: 1 and 2) set out in Figure 1 of Gray et al., *supra*, with the exceptions noted for $rBPI_{23}$ and with the exception that residue 417 is alanine (specified by GCT) rather than valine (specified by GTT). Other examples include dimeric forms of BPI fragments, as described in co-owned and co-pending U.S. Patent Application Serial No. 08/212,132, filed March 11, 1994, and corresponding PCT Application No. PCT/US95/03125, the disclosures of which are incorporated herein by reference. Preferred dimeric products include dimeric BPI protein products wherein the monomers are amino-terminal BPI fragments having the N-terminal residues from about 1 to 175 to about 1 to 199 of BPI holoprotein. A particularly preferred dimeric product is the dimeric form of the BPI fragment having N-terminal residues 1 through 193, designated $rBPI_{42}$ dimer.

Biologically active variants of BPI (BPI variants) include but are not limited to recombinant hybrid fusion proteins, comprising BPI holoprotein or biologically active fragment thereof and at least a portion of at least one other polypeptide, and dimeric forms of BPI variants. Examples of such hybrid fusion proteins and dimeric forms are described by Theofan et al. in co-owned, copending U.S. Patent Application Serial No. 07/885,911, and a continuation-in-part application thereof, U.S. Patent Application Serial No. 08/064,693 filed May 19, 1993 and corresponding PCT Application No. US93/04754 filed May 19, 1993, which are all incorporated herein by reference and include hybrid fusion proteins comprising, at the amino-terminal end, a BPI protein or a biologically active fragment thereof and, at the carboxy-terminal end, at least one constant domain of an immunoglobulin heavy chain or allelic variant thereof. Similarly configured hybrid fusion proteins involving part or all Lipopolysaccharide Binding Protein (LBP) are also contemplated for use in the present invention.

Biologically active analogs of BPI (BPI analogs) include but are not limited to BPI protein products wherein one or more amino acid residues have been replaced by a different amino acid. For example, co-owned, copending U.S. Patent Application Ser. No. 08/013,801 filed February 2, 1993 and corresponding PCT Application No. US94/01235 filed February 2, 1994, the disclosures of which are incorporated herein by reference, discloses polypeptide analogs of BPI and BPI

fragments wherein a cysteine residue is replaced by a different amino acid. A preferred BPI protein product described by this application is the expression product of DNA encoding from amino acid 1 to approximately 193 or 199 of the N-terminal amino acids of BPI holoprotein, but wherein the cysteine at residue number 132 is substituted with alanine and is designated $rBPI_{21}\Delta cys$ or $rBPI_{21}$. Other examples include dimeric forms of BPI analogs; e.g. co-owned and co-pending U.S. Patent Application Serial No. 08/212,132 filed March 11, 1994, and corresponding PCT Application No. PCT/US95/03125, the disclosures of which are incorporated herein by reference.

Other BPI protein products useful according to the methods of the invention are peptides derived from or based on BPI produced by recombinant or synthetic means (BPI-derived peptides), such as those described in co-owned and co-pending U.S. Patent Application Serial No. 08/504,841 filed July 20, 1995 and in co-owned and copending PCT Application No. PCT/US94/10427 filed September 15, 1994, which corresponds to U.S. Patent Application Serial No. 08/306,473 filed September 15, 1994, and PCT Application No. US94/02465 filed March 11, 1994, which corresponds to U.S. Patent Application Serial No. 08/209,762, filed March 11, 1994, which is a continuation-in-part of U.S. Patent Application Serial No. 08/183,222, filed January 14, 1994, which is a continuation-in-part of U.S. Patent Application Ser. No. 08/093,202 filed July 15, 1993 (for which the corresponding international application is PCT Application No. US94/02401 filed March 11, 1994), which is a continuation-in-part of U.S. Patent Application Ser. No. 08/030,644 filed March 12, 1993, the disclosures of all of which are incorporated herein by reference.

Presently preferred BPI protein products include recombinantly-produced N-terminal fragments of BPI, especially those having a molecular weight of approximately between 21 to 25 kD such as $rBPI_{23}$ or $rBPI_{21}$, or dimeric forms of these N-terminal fragments (e.g., $rBPI_{42}$ dimer). Additionally, preferred BPI protein products include $rBPI_{50}$ and BPI-derived peptides.

The administration of BPI protein products is preferably accomplished with a pharmaceutical composition comprising a BPI protein product and a pharmaceutically acceptable diluent, adjuvant, or carrier. The BPI protein product may be administered without or in conjunction with known surfactants, other

chemotherapeutic agents or additional known anti-microbial agents. One pharmaceutical composition containing BPI protein products (e.g., $rBPI_{50}$, $rBPI_{23}$) comprises the BPI protein product at a concentration of 1 mg/ml in citrate buffered saline (5 or 20 mM citrate, 150 mM NaCl, pH 5.0) comprising 0.1% by weight of poloxamer 188 (Pluronic F-68, BASF Wyandotte, Parsippany, NJ) and 0.002% by weight of polysorbate 80 (Tween 80, ICI Americas Inc., Wilmington, DE). Another pharmaceutical composition containing BPI protein products (e.g., $rBPI_{21}$) comprises the BPI protein product at a concentration of 2 mg/mL in 5 mM citrate, 150 mM NaCl, 0.2% poloxamer 188 and 0.002% polysorbate 80. Such combinations are described in co-owned, co-pending PCT Application No. US94/01239 filed February 2, 1994, which corresponds to U.S. Patent Application Ser. No. 08/190,869 filed February 2, 1994 and U.S. Patent Application Ser. No. 08/012,360 filed February 2, 1993, the disclosures of all of which are incorporated herein by reference.

Other aspects and advantages of the present invention will be understood upon consideration of the following illustrative examples. Example 1 addresses the effect of BPI protein product administration on mortality associated with meningococcemia. Example 2 addresses the effect of BPI protein product administration on morbidities associated with meningococcemia. Examples 3 and 4 describe the effect of BPI protein product administration on the course of meningococcemia in two particular individuals.

Example 1

Clinical Study Protocol - Effect of BPI Protein Product on Mortality

A human clinical study was designed to examine the effect of an exemplary BPI protein product, $rBPI_{21}$, on clinical outcome in pediatric patients suffering from severe systemic meningococcal disease. Clinical outcomes (mortality, amputations, grafts, permanent neurologic impairment) were assessed through study day 28 or discharge, whichever occurred first. Additionally, the safety, pharmacokinetics and hemodynamic effects of the BPI protein product were assessed.

Thus, a Phase I open-label multicenter study of the effects of BPI

protein product on pediatric patients with severe meningococcemia receiving standard care was implemented. Patients who met eligibility criteria were enrolled following informed consent of the parent or legal guardian. The eligibility criteria were such that the patients enrolled had a 90% predicted rate of severe adverse outcome, defined as death, stroke, amputation, or skin grafting. All patients received comprehensive pediatric intensive care consistent with the usual standard of care, and received their first dose of antibiotics no more than 8 hours prior to the beginning of BPI protein product administration.

The first four patients received an infusion of 0.5 mg/kg $rBPI_{21}$ over 30 minutes, followed immediately by a continuous infusion of $rBPI_{21}$ at a rate of 0.5 mg/kg/day for 24 hours. The next six patients received an infusion of 1.0 mg/kg $rBPI_{21}$ over 30 minutes, followed immediately by a continuous infusion of $rBPI_{21}$ at a rate of 1.0 mg/kg/day for 24 hours. The remaining patients received an infusion of 2.0 mg/kg $rBPI_{21}$ over 30 minutes, followed immediately by a continuous infusion of $rBPI_{21}$ at a rate of 2.0 mg/kg/day for 24 hours. All study centers escalated to the higher dose levels at the same time.

The pharmacokinetics of the BPI protein product and circulating endotoxin levels were assessed by serial monitoring of plasma for $rBPI_{21}$ and endotoxin by limulus amoebocyte lysate (LAL) assay. Any acute hemodynamic effects associated with administration of $rBPI_{21}$ were described by recording standard hemodynamic parameters, including: heart rate, invasive systemic arterial blood pressure, electrocardiogram, oxygen saturation, and invasive hemodynamic measurements obtained from a pulmonary artery catheter. No new invasive devices were placed for the purposes of the study; the placement of medical devices was at the sole discretion of the attending physician and his/her staff, and only for the purpose of monitoring the patient consistent with normal standards of care.

Safety was monitored by continuous measurements of vital signs and hemodynamics, physical examinations and pre- and post-treatment safety laboratory assessments. Patients were followed for safety until death, hospital discharge or study day 28, whichever occurred first.

Patients with severe meningococcemia were selected for enrollment in the study if they met the following inclusion and exclusion criteria. Inclusion criteria

were: (1) age 1 year to 18 years inclusive; (2) presumptive diagnosis of meningococcemia, based on any or all of the following: (a) petechiae or purpura, fever, and hemodynamic instability in a clinical context consistent with the diagnosis of meningococcemia, (b) demonstration of gram-negative diplococci in blood, cerebrospinal fluid, or skin lesions in a clinical context consistent with the diagnosis of meningococcemia, and/or (c) demonstration of meningococcal antigens by immunologic determination in a clinical context consistent with the diagnosis of meningococcemia; (3) Glasgow Meningococcal Septicemia Prognostic Score of 8 or greater [Sinclair et al., *supra*]; (4) patient history of having received the first dose of antibiotics no more than 8 hours prior to beginning BPI protein product administration; (5) negative pregnancy test for pubertal or post-pubertal females; (6) written informed consent obtained from the parent or legal guardian; and (7) collection of confidential patient follow-up information. Exclusion criteria were: (1) insufficient vascular access to administer BPI protein product without compromising routine ICU care; (2) exposure to investigational agents during the last 30 days prior to study entry; and (3) any condition that in the attending physician's judgment would make the patient unsuitable for participation in the study, including imminent mortality.

The following were performed within 24 hours prior to enrollment in the study: (1) medical history, (2) complete physical examination, (3) chest x-ray, (4) laboratory evaluation: Hematology: CBC, differential; Coagulation: PT, PTT, fibrinogen, D-Dimers; Microbiology: cultures, Gram stains, serology as indicated; Chemistries: sodium, potassium, chloride, bicarbonate, glucose, BUN, creatinine, ionized calcium, phosphorus, magnesium, bilirubin, AST, ALT, CPK (with isoenzymes), LDH; Arterial Blood Gases; Urinalysis: chemistry and microscopic; and (5) procurement of written informed consent and collection of confidential follow-up information.

The $rBPI_{21}$ was supplied as a clear, colorless, sterile non-pyrogenic solution in 10 mL single use glass vials at a concentration of 2 mg/mL in 5 mM sodium citrate/0.15 M sodium chloride buffer, pH 5.0 with 0.2% poloxamer 188 and 0.002% polysorbate 80 containing no preservative. For storage, the $rBPI_{21}$ vial was refrigerated at 2-8°C at all times prior to administration. The product was brought

to room temperature prior to infusion, and was administered via a central vein or other suitable vein. Suitability of intravenous access was determined by easy withdrawal of blood from the access, as well as easy infusion of intravenous fluids without infiltration. $rBPI_{21}$ was the sole agent administered in the chosen port during the course of the infusion protocol. The venous access port was not heparinized, but was flushed as necessary with physiologic saline.

After BPI protein product infusion had started, patients were observed for the possible development of adverse events. Plasma samples for determination of $rBPI_{21}$ levels were collected immediately prior to the start of the infusion (time zero) and at the following times after the start of the infusion: 30 min., 90 min., 240 min., 720 min., just prior to termination of infusion at 24 hours 30 min., 24 hours 37 min., 24 hours 45 min., 25 hours, 25 hours 30 min., 26 hours 30 min., 27 hours 30 min., and 48 hours. Plasma samples for determination of endotoxin levels were drawn immediately prior to the onset of the infusion (time zero) and at the following times after the start of the infusion: 30 min., 90 min., 240 min., 720 min. and at 48 hours. Serum ionized calcium concentrations were determined immediately prior to the onset of the infusion (time zero) and at the following times after the start of infusion: 30 min., 2 hours, 6 hours, 12 hours, and 24 hours. Monitoring the ionized calcium concentrations is the usual standard of care in meningococcemia and normally occurs every 4 hours. All samples were obtained via a line not used to infuse BPI protein product.

The following vital signs were recorded every 5 min. for thirty min. prior to beginning the infusion, every 5 min. during the 30-min. loading dose, and every 30 min. thereafter for 24 hours: (a) heart rate; (b) systemic arterial blood pressures: systolic, diastolic, and mean; and (c) respiratory rate (if the patient was spontaneously breathing). In addition to the manual collection as outlined above, data was digitally recorded and stored every minute within the bedside monitor during the ICU stay. Once the patient left the ICU, vital signs were collected daily until hospital discharge.

The following invasive hemodynamic parameters were recorded every 10 min. for the 30 min. prior to beginning the infusion, every 10 min. during the 30-min. loading dose, and every 2 hours thereafter for 24 hours: (a) mixed venous

oxygen saturation (oximetric catheters only); (b) pulmonary artery wedge pressure; (c) pulmonary artery pressures: systolic, diastolic, mean; (d) cardiac index (CI); (e) systemic vascular resistance index (SVRI); (f) pulmonary vascular resistance index (PVRI); and (g) stroke volume index (SVI). A complete profile of medications and vasoactive infusions was recorded through hospital discharge. The following were documented with a frequency determined by the primary care physician consistent with standard management of severe meningococcal disease: (a) arterial blood gases, (b) venous blood gases, (c) oxygen delivery (DO_2), (d) oxygen consumption (VO_2), (e) hematology, (f) coagulation, and (g) blood chemistries. The PRISM Score (Pediatric Risk of Mortality Score) was also calculated and recorded at the end of the first hospital day. Table 1 below shows the factors with the corresponding number of points used to calculate the PRISM score (Pollack et al., "The pediatric risk of mortality (PRISM) score," *Critical Care Medicine* 16:1110, 1988).

Table 1
Pediatric Risk of Mortality Score
(PRISM Score)

FACTORS	AGE RESTRICTION AND RANGES			Pts
Systolic BP (mm/Hg)	**Infants only**	**Children only**	**All Ages**	
	130 - 160	150 - 200		2
	55 - 65	65 - 75		2
	> 160	> 200		6
	40 - 54	50 - 64		6
	< 40	< 50		7
Diastolic BP (mm Hg)			> 110	6
Heart Rate (beats/min)	> 160	>150		4
	< 90	< 70		4
Respiratory Rate (breaths/min)	61 - 90	51 - 70		1
	> 90	> 70		5
	APNEA	APNEA		5
PaO_2/FiO_2			200 - 300	2
			< 200	3
$PaCO_2$ (mm Hg)			51 - 65	1
			> 65	5
Glasgow score			< 8	6
Pupillary Reactions			Unequal or dilated	4
			Fixed and dilated	10
PT/PTT			> 1.5 x Control	2
Total Bilirubin (mg/dl)			> 3.5 at age > 1 month	6
Potassium (meq/l)			3.0 - 3.5	1
			6.5 - 7.5	1
			< 3.0	5
			> 7.5	5

Table 1
Pediatric Risk of Mortality Score
(PRISM Score)

FACTORS	AGE RESTRICTION AND RANGES			Pts
Calcium (mg/dl)			7.0 - 8.0	2
			12.0 - 15.0	2
			< 7.0	6
			> 15.0	6
Glucose (mg/dl)			40 - 60	4
			250 - 400	4
			< 40	8
			> 400	8
Bicarbonate (meq/l)			< 16	3
			> 32	3

At the end of the study (i.e., study day 28 or at time of discharge, whichever occurred first), a physical exam, including vital signs, and a review of any adverse events were performed. The following clinical outcomes were also assessed: (a) mortality; (b) amputations; (c) grafting procedures; (d) permanent neurologic impairment including but not limited to cerebrovascular accidents, cerebral atrophy, and seizures requiring medication that manifested as impaired neurologic function; and (e) pediatric outcome scores (based on the Pediatric Cerebral Performance Category Scale and/or the Pediatric Overall Performance Category Scale, as described by Fiser, "Assessing the outcome of pediatric intensive care," *J. Pediatrics* 121:1 68-74, 1992).

A review was conducted of the medical records of patients admitted to one participating clinical center, Study Center 1, during the two years immediately prior to the initiation of the BPI study. From these records, 14 children were selected for comparative analysis because they met the first three above-described inclusion criteria regarding age, presumptive meningococcemia diagnosis and Glasgow score. Six of these 14 "historical control" children died. This high mortality rate was expected, considering that the inclusion criteria required a Glasgow score of 8 or greater. A Glasgow score of 8 or greater always indicates severe disease.

In striking contrast, none of the 10 patients in the BPI study at Study Center 1 died. Thus, the administration of BPI protein product dramatically reduced the mortality rate of severe pediatric meningococcemia at Study Center 1 from 43% to 0%. When results from all of the participating clinical centers are included, of the 14 total patients enrolled in the BPI study so far, only one patient has died -- a mortality rate of only 7%. This low mortality rate is particularly remarkable considering that 12 of the 14 patients had a Glasgow score of 10 or greater when they entered the study. Multiple analyses that calculated expected mortality for the 14 patients in the BPI study, based on indicators (levels of endotoxin, TNF, IL-6 and fibrinogen, and PTT) that have been shown to correlate with disease severity and outcome in various studies [Brandtzaeg et al., *supra*, Van Deuren et al., *supra*, McManus et al., *supra*, and Bone, *supra*] predicted a mortality rate ranging from about 20% to about 50% for this population. Endotoxin, TNF and IL-6 levels for the initial 10 patients in the BPI study are shown in Figures 1-6.

EXAMPLE 2

Effect of BPI Protein Product on Morbidity

The clinical outcomes of patients treated in accordance with the BPI protein product study protocol described in Example 1 above are summarized in Table 2 below and compared with the clinical outcomes of the 14 "historical control" children at Study Center 1 who would have met the study's inclusion criteria during the two years immediately prior to the initiation of the study. The natural history of the clinical course of meningococcemia would have been largely similar; previously healthy children underwent a 12-24 hour flu-like prodrome, developed purpura, and then died or became moribund within 4-6 hours. Typically, such meningococcemia patients continue to become sicker in the PICU, at least for the first 12 hours, and often succumb to irreversibly progressive shock. There were no known differences in the standard of care provided to the patients enrolled in the BPI study compared to the 14 previous "historical control" patients at Study Center 1, with the exception of BPI protein product administration.

Table 2	"Historical Control" Children at Study Center 1	BPI Study Children at Study Center 1	BPI Study Children at All Centers
No. Satisfying Inclusion Criteria or Enrolled in BPI Study	14	10	14
No. of Deaths	6 (43%)	0 (0%)	1 (7%)
Morbidities			
No. of Survivors With Severe Amputations	2/8 (25%)	1/10 (10%)	1/13 (8%)
No. of Survivors With Permanent Neurologic Impairment	2/8 (25%)	0/10 (0%)	0/13 (0%)
Total Morbidity Events (severe amputation or permanent neurologic impairment)	4/8* (50%)	1/10 (10%)	1/13 (8%)

* One patient experienced both severe amputations and permanent neurologic impairment.

The results summarized in Table 2 show that administration of BPI protein product not only vastly reduced the mortality rate at Study Center 1, but also reduced the incidence of severe morbidities from 50% to 10% at Study Center 1. When results from all of the participating clinical centers are included, the overall severe morbidity rate remains low, at 8%. Interpretation of morbidity data from this study is somewhat complicated by the fact that BPI protein product treatment had a significant effect on reducing the number of mortalities associated with this disease, that is, a number of the severely ill patients were rescued who would have otherwise succumbed to the disease. No analysis was performed to predict the morbidities patients would have experienced had they not died. For this morbidity outcome analysis, amputations at the wrist or above, or at the ankle or above, were considered to be severe amputations. Neurologic abnormalities that resulted in significant and permanent impairment of motor, cognitive or sensory function were considered to be permanent neurologic impairments. Four additional BPI study patients (all at Study

Center 1) experienced minor amputations of toes or fingers (and one partial foot amputation). Four additional "historical control" patients at Study Center 1 experienced other neurologic abnormalities, including cerebrovascular accidents, seizures, CT scan or EEG abnormalities, and cranial nerve palsy. One BPI study patient (at Study Center 1) experienced a neurologic abnormality (see Example 4).

EXAMPLE 3

Clinical Course of One Individual in the BPI Study

This patient (number 2 in the BPI study) was a seven year old white male who was previously healthy. On the night prior to admission to the pediatric intensive care unit (PICU), he came to the emergency room with symptoms of fever, vomiting, and headache. His white blood cell count (WBC) was 17,500, but he was sent home because his exam was not suggestive of significant disease. He was seen again the next morning, when a diffuse petechial rash was noted. His WBC had dropped to 6,300, and his fever and headache had worsened. Meningococcemia was suspected, and after initial fluid infusion and antibiotic administration, he was admitted to the Pediatric Intensive Care Unit (PICU) at Study Center 1.

He was intubated and mechanically ventilated, fluid resuscitated, and begun on inotropic support with an epinephrine infusion. He was treated with the antibiotic ceftriaxone. Before enrollment in the BPI study, his Glasgow score was 14/15, his PRISM score was 17, and his physical exam revealed a temperature of 38.8°C, a heart rate of 145, a respiratory rate of 16 on the ventilator, and a blood pressure of 107/46. His PTT value was 25.2 and his fibrinogen level was 480. Blood cultures revealed *N. meningitidis* serotype C. A pulmonary artery catheter was placed for monitoring, and written informed consent was obtained for the BPI protocol described above in Example 1.

An $rBPI_{21}$ infusion was started at 14:35 on the day of admission to PICU (Day One). He received a dose of 0.5 mg/kg $rBPI_{21}$ over 30 minutes followed by an infusion of 0.5 mg/kg over the following 24 hours. He tolerated his infusion well with no hemodynamic changes or other complications. His PICU course was relatively uneventful; his inotropic support was discontinued on the night of Day Four, after which his ventilator was rapidly weaned. He was taken off the ventilator

during Day Five without any problems. He was transferred to the general pediatric wards on the afternoon of Day Six and discharged from the hospital on Day Nine without any complications.

EXAMPLE 4

Clinical Course of Another Individual in the BPI Study

This patient (number 6 in the BPI study) was an 18 year-old white male who was previously in excellent health. Prior to admission, he experienced a two-day history of sore throat and lethargy. On the evening of admission, his roommate found him in the corner of his dormitory room, unresponsive and covered with a purple petechial rash. He was transported via ambulance to the hospital, at which time his temperature was 101°F and he was in fulminant shock. He was treated with fluid resuscitation, ceftriaxone antibiotic, and a dopamine infusion to improve circulation. He was transported via helicopter to Study Center 1.

On arrival, he was intubated and ventilated. Before enrollment in the BPI study, his Glasgow score was 12/15 and his PRISM score was 16. He was moribund. His physical exam was significant for rapidly expanding diffuse purpura, a capillary refill greater than eight seconds, and minimally detectable pulses. His feet were blue, cold, and pulseless, as were all ten fingers. He had a temperature of 37.7°C, a heart rate of 150, a respiratory rate of 20 on the ventilator, and a blood pressure of 133/66. His laboratory evaluation was significant for a WBC of 7,500 with a differential of 73% segs and 14% bands. His PT was 26, his PTT was greater than 114, his fibrinogen level was 121, and his D-dimers were greater than 8. Cerebrospinal fluid cultures revealed *N. meningitidis* serotype C. He had biochemical evidence of multi-organ system failure on arrival. Fluid resuscitation continued and an epinephrine infusion was begun. Informed consent was obtained and he was enrolled in the BPI study according to Example 1 above.

An $rBPI_{21}$ infusion at a dose of 1 mg/kg over 30 minutes was begun at 07:15 on the day of his admission to PICU (Day One), followed by a dose of 1 mg/kg over the next 24 hours. The $rBPI_{21}$ infusions were well tolerated without adverse hemodynamic effects. He required hemodynamic support with inotropic infusions of dopamine, dobutamine and epinephrine. He was weaned off of

epinephrine and dopamine on the morning of Day Three, and rapid weaning of his dobutamine followed. Invasive evaluation of his hemodynamic status with a Swan-Ganz catheter revealed a hyperdynamic state which did not undergo transition to hypodynamic state, as would be typical of such severely ill children on day two of therapy. Although his coagulopathy was initially severe and required multiple transfusions of fresh frozen plasma, packed cells, and platelets, the coagulopathy had rapidly resolved by Day Three of hospitalization. Also by Day Three, his initial cold and unperfused feet began to return to a normal pink color, with evidence of warmth and circulation being restored. Additional antibiotic therapy with vancomycin and tobramycin was begun. On Day Four his clinical status had improved to the point that his ventilator was weaned. The ventilatory wean continued throughout that day, but this wean was interrupted on Day Five by transient pulmonary edema. This pulmonary edema was believed due to resorption of third spaced fluid and healing of his vascular leak. His ventilator wean was continued later on Day Five. His circulation in his lower extremities had markedly improved to the point that upon his discharge from PICU on Day Eight, tissue injury was only evident in his left heel, which was debrided, and his left second toe, which was ultimately amputated. During his hospital course, following an episode of transient hypertension, he underwent a CT-scan which revealed evidence of a right temporal/parietal cerebrovascular accident (CVA) which dated approximately to the date of his admission to PICU. The CVA was neurologically silent and did not compromise any motor, cognitive, or sensory functions. His post-PICU stay on the pediatric ward consisted primarily of physical therapy, occupational therapy, and enhanced nutrition. He was discharged on Day Fourteen to a rehabilitation facility for further work on strengthening and general rehabilitation.

Numerous modifications and variations of the above-described invention are expected to occur to those of skill in the art. Accordingly, only such limitations as appear in the appended claims should be placed thereon.

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(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

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(ix) TELECOMMUNICATION INFORMATION:
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(B) TELEFAX: 312/474-0448
(C) TELEX: 25-3856

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1813 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 31..1491

(ix) FEATURE:
(A) NAME/KEY: mat_peptide

(B) LOCATION: 124..1491

(ix) FEATURE:
(A) NAME/KEY: misc_feature

(D) OTHER INFORMATION: "rBPI"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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CAGGCCTTGA GGTTTTGGCA GCTCTGGAGG ATG AGA GAG AAC ATG GCC AGG GGC        54
                                 Met Arg Glu Asn Met Ala Arg Gly
                                 -31 -30                 -25

CCT TGC AAC GCG CCG AGA TGG GTG TCC CTG ATG GTG CTC GTC GCC ATA       102
Pro Cys Asn Ala Pro Arg Trp Val Ser Leu Met Val Leu Val Ala Ile
            -20                 -15                 -10

GGC ACC GCC GTG ACA GCG GCC GTC AAC CCT GGC GTC GTG GTC AGG ATC       150
Gly Thr Ala Val Thr Ala Ala Val Asn Pro Gly Val Val Val Arg Ile
        -5                  1                   5

TCC CAG AAG GGC CTG GAC TAC GCC AGC CAG CAG GGG ACG GCC GCT CTG       198
Ser Gln Lys Gly Leu Asp Tyr Ala Ser Gln Gln Gly Thr Ala Ala Leu
 10                  15                  20                  25

CAG AAG GAG CTG AAG AGG ATC AAG ATT CCT GAC TAC TCA GAC AGC TTT       246
Gln Lys Glu Leu Lys Arg Ile Lys Ile Pro Asp Tyr Ser Asp Ser Phe
                30                  35                  40

AAG ATC AAG CAT CTT GGG AAG GGG CAT TAT AGC TTC TAC AGC ATG GAC       294
Lys Ile Lys His Leu Gly Lys Gly His Tyr Ser Phe Tyr Ser Met Asp
            45                  50                  55

ATC CGT GAA TTC CAG CTT CCC AGT TCC CAG ATA AGC ATG GTG CCC AAT       342
Ile Arg Glu Phe Gln Leu Pro Ser Ser Gln Ile Ser Met Val Pro Asn
        60                  65                  70

GTG GGC CTT AAG TTC TCC ATC AGC AAC GCC AAT ATC AAG ATC AGC GGG       390
Val Gly Leu Lys Phe Ser Ile Ser Asn Ala Asn Ile Lys Ile Ser Gly
    75                  80                  85

AAA TGG AAG GCA CAA AAG AGA TTC TTA AAA ATG AGC GGC AAT TTT GAC       438
Lys Trp Lys Ala Gln Lys Arg Phe Leu Lys Met Ser Gly Asn Phe Asp
 90                  95                  100                 105

CTG AGC ATA GAA GGC ATG TCC ATT TCG GCT GAT CTG AAG CTG GGC AGT       486
Leu Ser Ile Glu Gly Met Ser Ile Ser Ala Asp Leu Lys Leu Gly Ser
                110                 115                 120

AAC CCC ACG TCA GGC AAG CCC ACC ATC ACC TGC TCC AGC TGC AGC AGC       534
Asn Pro Thr Ser Gly Lys Pro Thr Ile Thr Cys Ser Ser Cys Ser Ser
            125                 130                 135

CAC ATC AAC AGT GTC CAC GTG CAC ATC TCA AAG AGC AAA GTC GGG TGG       582
His Ile Asn Ser Val His Val His Ile Ser Lys Ser Lys Val Gly Trp
        140                 145                 150

CTG ATC CAA CTC TTC CAC AAA AAA ATT GAG TCT GCG CTT CGA AAC AAG       630
Leu Ile Gln Leu Phe His Lys Lys Ile Glu Ser Ala Leu Arg Asn Lys
    155                 160                 165

ATG AAC AGC CAG GTC TGC GAG AAA GTG ACC AAT TCT GTA TCC TCC AAG       678
Met Asn Ser Gln Val Cys Glu Lys Val Thr Asn Ser Val Ser Ser Lys
170                 175                 180                 185

CTG CAA CCT TAT TTC CAG ACT CTG CCA GTA ATG ACC AAA ATA GAT TCT       726
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Leu Gln Pro Tyr Phe Gln Thr Leu Pro Val Met Thr Lys Ile Asp Ser
                190                 195                 200

GTG GCT GGA ATC AAC TAT GGT CTG GTG GCA CCT CCA GCA ACC ACG GCT      774
Val Ala Gly Ile Asn Tyr Gly Leu Val Ala Pro Pro Ala Thr Thr Ala
            205                 210                 215

GAG ACC CTG GAT GTA CAG ATG AAG GGG GAG TTT TAC AGT GAG AAC CAC      822
Glu Thr Leu Asp Val Gln Met Lys Gly Glu Phe Tyr Ser Glu Asn His
        220                 225                 230

CAC AAT CCA CCT CCC TTT GCT CCA CCA GTG ATG GAG TTT CCC GCT GCC      870
His Asn Pro Pro Pro Phe Ala Pro Pro Val Met Glu Phe Pro Ala Ala
    235                 240                 245

CAT GAC CGC ATG GTA TAC CTG GGC CTC TCA GAC TAC TTC TTC AAC ACA      918
His Asp Arg Met Val Tyr Leu Gly Leu Ser Asp Tyr Phe Phe Asn Thr
250                 255                 260                 265

GCC GGG CTT GTA TAC CAA GAG GCT GGG GTC TTG AAG ATG ACC CTT AGA      966
Ala Gly Leu Val Tyr Gln Glu Ala Gly Val Leu Lys Met Thr Leu Arg
                270                 275                 280

GAT GAC ATG ATT CCA AAG GAG TCC AAA TTT CGA CTG ACA ACC AAG TTC     1014
Asp Asp Met Ile Pro Lys Glu Ser Lys Phe Arg Leu Thr Thr Lys Phe
            285                 290                 295

TTT GGA ACC TTC CTA CCT GAG GTG GCC AAG AAG TTT CCC AAC ATG AAG     1062
Phe Gly Thr Phe Leu Pro Glu Val Ala Lys Lys Phe Pro Asn Met Lys
        300                 305                 310

ATA CAG ATC CAT GTC TCA GCC TCC ACC CCG CCA CAC CTG TCT GTG CAG     1110
Ile Gln Ile His Val Ser Ala Ser Thr Pro Pro His Leu Ser Val Gln
    315                 320                 325

CCC ACC GGC CTT ACC TTC TAC CCT GCC GTG GAT GTC CAG GCC TTT GCC     1158
Pro Thr Gly Leu Thr Phe Tyr Pro Ala Val Asp Val Gln Ala Phe Ala
330                 335                 340                 345

GTC CTC CCC AAC TCC TCC CTG GCT TCC CTC TTC CTG ATT GGC ATG CAC     1206
Val Leu Pro Asn Ser Ser Leu Ala Ser Leu Phe Leu Ile Gly Met His
                350                 355                 360

ACA ACT GGT TCC ATG GAG GTC AGC GCC GAG TCC AAC AGG CTT GTT GGA     1254
Thr Thr Gly Ser Met Glu Val Ser Ala Glu Ser Asn Arg Leu Val Gly
            365                 370                 375

GAG CTC AAG CTG GAT AGG CTG CTC CTG GAA CTG AAG CAC TCA AAT ATT     1302
Glu Leu Lys Leu Asp Arg Leu Leu Leu Glu Leu Lys His Ser Asn Ile
        380                 385                 390

GGC CCC TTC CCG GTT GAA TTG CTG CAG GAT ATC ATG AAC TAC ATT GTA     1350
Gly Pro Phe Pro Val Glu Leu Leu Gln Asp Ile Met Asn Tyr Ile Val
    395                 400                 405

CCC ATT CTT GTG CTG CCC AGG GTT AAC GAG AAA CTA CAG AAA GGC TTC     1398
Pro Ile Leu Val Leu Pro Arg Val Asn Glu Lys Leu Gln Lys Gly Phe
410                 415                 420                 425

CCT CTC CCG ACG CCG GCC AGA GTC CAG CTC TAC AAC GTA GTG CTT CAG     1446
Pro Leu Pro Thr Pro Ala Arg Val Gln Leu Tyr Asn Val Val Leu Gln
                430                 435                 440

CCT CAC CAG AAC TTC CTG CTG TTC GGT GCA GAC GTT GTC TAT AAA         1491
Pro His Gln Asn Phe Leu Leu Phe Gly Ala Asp Val Val Tyr Lys
            445                 450                 455
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TGAAGGCACC AGGGGTGCCG GGGGCTGTCA GCCGCACCTG TTCCTGATGG GCTGTGGGGC 1551

ACCGGCTGCC TTTCCCCAGG GAATCCTCTC CAGATCTTAA CCAAGAGCCC CTTGCAAACT 1611

TCTTCGACTC AGATTCAGAA ATGATCTAAA CACGAGGAAA CATTATTCAT TGGAAAAGTG 1671

CATGGTGTGT ATTTTAGGGA TTATGAGCTT CTTTCAAGGG CTAAGGCTGC AGAGATATTT 1731

CCTCCAGGAA TCGTGTTTCA ATTGTAACCA AGAAATTTCC ATTTGTGCTT CATGAAAAAA 1791

AACTTCTGGT TTTTTTCATG TG 1813

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 487 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Arg Glu Asn Met Ala Arg Gly Pro Cys Asn Ala Pro Arg Trp Val
-31 -30 -25 -20

Ser Leu Met Val Leu Val Ala Ile Gly Thr Ala Val Thr Ala Ala Val
-15 -10 -5 1

Asn Pro Gly Val Val Val Arg Ile Ser Gln Lys Gly Leu Asp Tyr Ala
5 10 15

Ser Gln Gln Gly Thr Ala Ala Leu Gln Lys Glu Leu Lys Arg Ile Lys
20 25 30

Ile Pro Asp Tyr Ser Asp Ser Phe Lys Ile Lys His Leu Gly Lys Gly
35 40 45

His Tyr Ser Phe Tyr Ser Met Asp Ile Arg Glu Phe Gln Leu Pro Ser
50 55 60 65

Ser Gln Ile Ser Met Val Pro Asn Val Gly Leu Lys Phe Ser Ile Ser
70 75 80

Asn Ala Asn Ile Lys Ile Ser Gly Lys Trp Lys Ala Gln Lys Arg Phe
85 90 95

Leu Lys Met Ser Gly Asn Phe Asp Leu Ser Ile Glu Gly Met Ser Ile
100 105 110

Ser Ala Asp Leu Lys Leu Gly Ser Asn Pro Thr Ser Gly Lys Pro Thr
115 120 125

Ile Thr Cys Ser Ser Cys Ser Ser His Ile Asn Ser Val His Val His
130 135 140 145

Ile Ser Lys Ser Lys Val Gly Trp Leu Ile Gln Leu Phe His Lys Lys
150 155 160

Ile Glu Ser Ala Leu Arg Asn Lys Met Asn Ser Gln Val Cys Glu Lys
165 170 175

Val Thr Asn Ser Val Ser Ser Lys Leu Gln Pro Tyr Phe Gln Thr Leu
180 185 190

Pro Val Met Thr Lys Ile Asp Ser Val Ala Gly Ile Asn Tyr Gly Leu

195 200 205

Val Ala Pro Pro Ala Thr Thr Ala Glu Thr Leu Asp Val Gln Met Lys
210 215 220 225

Gly Glu Phe Tyr Ser Glu Asn His His Asn Pro Pro Pro Phe Ala Pro
230 235 240

Pro Val Met Glu Phe Pro Ala Ala His Asp Arg Met Val Tyr Leu Gly
245 250 255

Leu Ser Asp Tyr Phe Phe Asn Thr Ala Gly Leu Val Tyr Gln Glu Ala
260 265 270

Gly Val Leu Lys Met Thr Leu Arg Asp Asp Met Ile Pro Lys Glu Ser
275 280 285

Lys Phe Arg Leu Thr Thr Lys Phe Phe Gly Thr Phe Leu Pro Glu Val
290 295 300 305

Ala Lys Lys Phe Pro Asn Met Lys Ile Gln Ile His Val Ser Ala Ser
310 315 320

Thr Pro Pro His Leu Ser Val Gln Pro Thr Gly Leu Thr Phe Tyr Pro
325 330 335

Ala Val Asp Val Gln Ala Phe Ala Val Leu Pro Asn Ser Ser Leu Ala
340 345 350

Ser Leu Phe Leu Ile Gly Met His Thr Thr Gly Ser Met Glu Val Ser
355 360 365

Ala Glu Ser Asn Arg Leu Val Gly Glu Leu Lys Leu Asp Arg Leu Leu
370 375 380 385

Leu Glu Leu Lys His Ser Asn Ile Gly Pro Phe Pro Val Glu Leu Leu
390 395 400

Gln Asp Ile Met Asn Tyr Ile Val Pro Ile Leu Val Leu Pro Arg Val
405 410 415

Asn Glu Lys Leu Gln Lys Gly Phe Pro Leu Pro Thr Pro Ala Arg Val
420 425 430

Gln Leu Tyr Asn Val Val Leu Gln Pro His Gln Asn Phe Leu Leu Phe
435 440 445

Gly Ala Asp Val Val Tyr Lys
450 455

WHAT IS CLAIMED IS:

1. A method of treating meningococcemia comprising the step of administering a therapeutically effective amount of a bactericidal/permeability-increasing (BPI) protein product to a human suffering from meningococcemia.

2. The method of claim 1 wherein the BPI protein product is an amino-terminal fragment of BPI protein having a molecular weight of about 21 kD to 25 kD.

3. The method of claim 1 wherein the BPI protein product is $rBPI_{23}$ or a dimeric form thereof.

4. The method of claim 1 wherein the BPI protein product is $rBPI_{21}$.

5. The method of claim 1 wherein the BPI protein product is concurrently administered with another therapeutic agent.

6. Use of a BPI protein product in the preparation of a medicament for the treatment of meningococcemia in humans.

7. The use according to claim 6 wherein the BPI protein product is selected from the group consisting of amino-terminal fragments of BPI protein having a molecular weight of about 21 kD to 25 kD, $rBPI_{23}$ or dimeric forms thereof, and $rBPI_{21}$.

FIG. 1

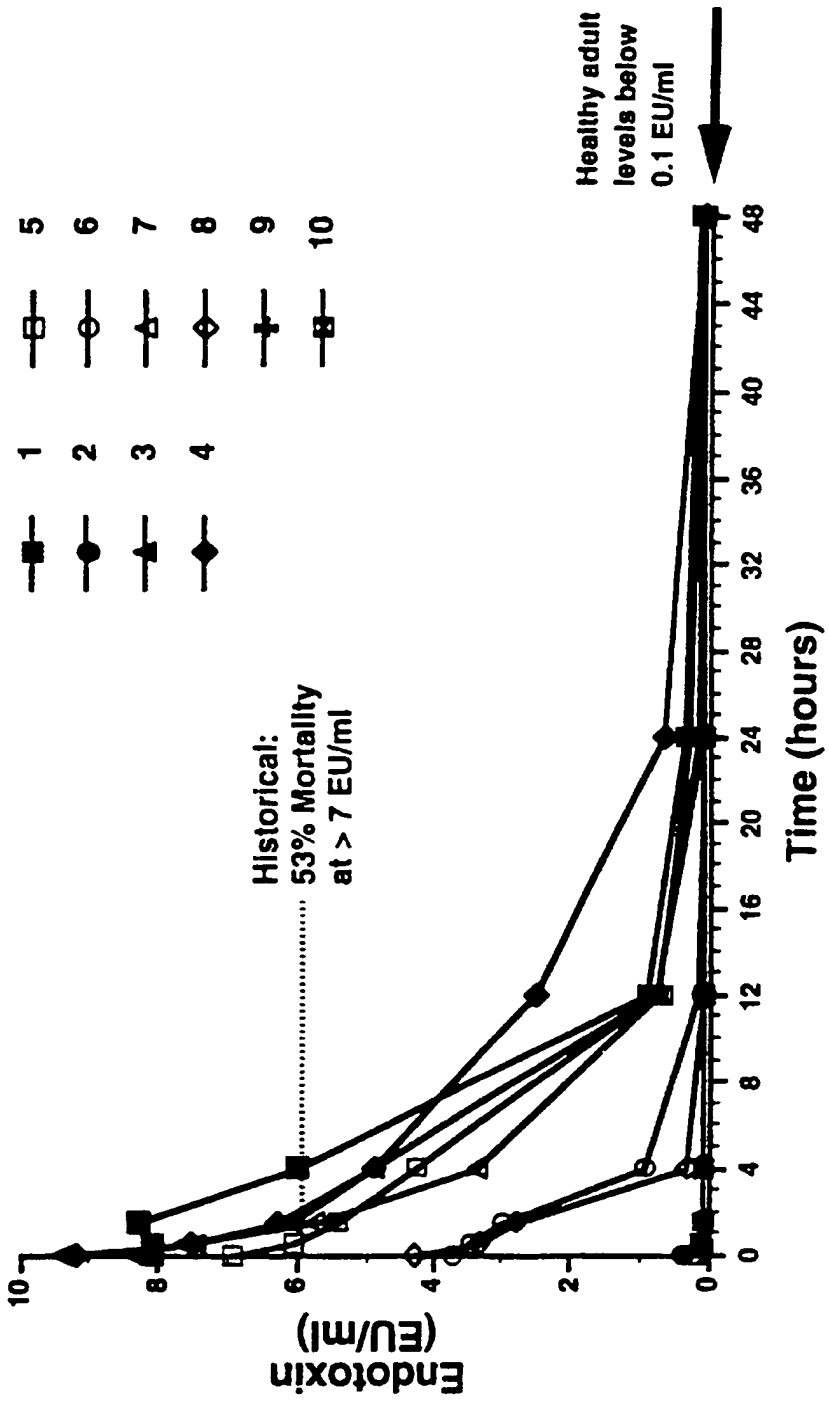

Endotoxin (EU/ml)
Time (hours)
Historical: 53% Mortality at > 7 EU/ml
Healthy adult levels below 0.1 EU/ml
1
2
3
4
5
6
7
8
9
10

FIG. 2

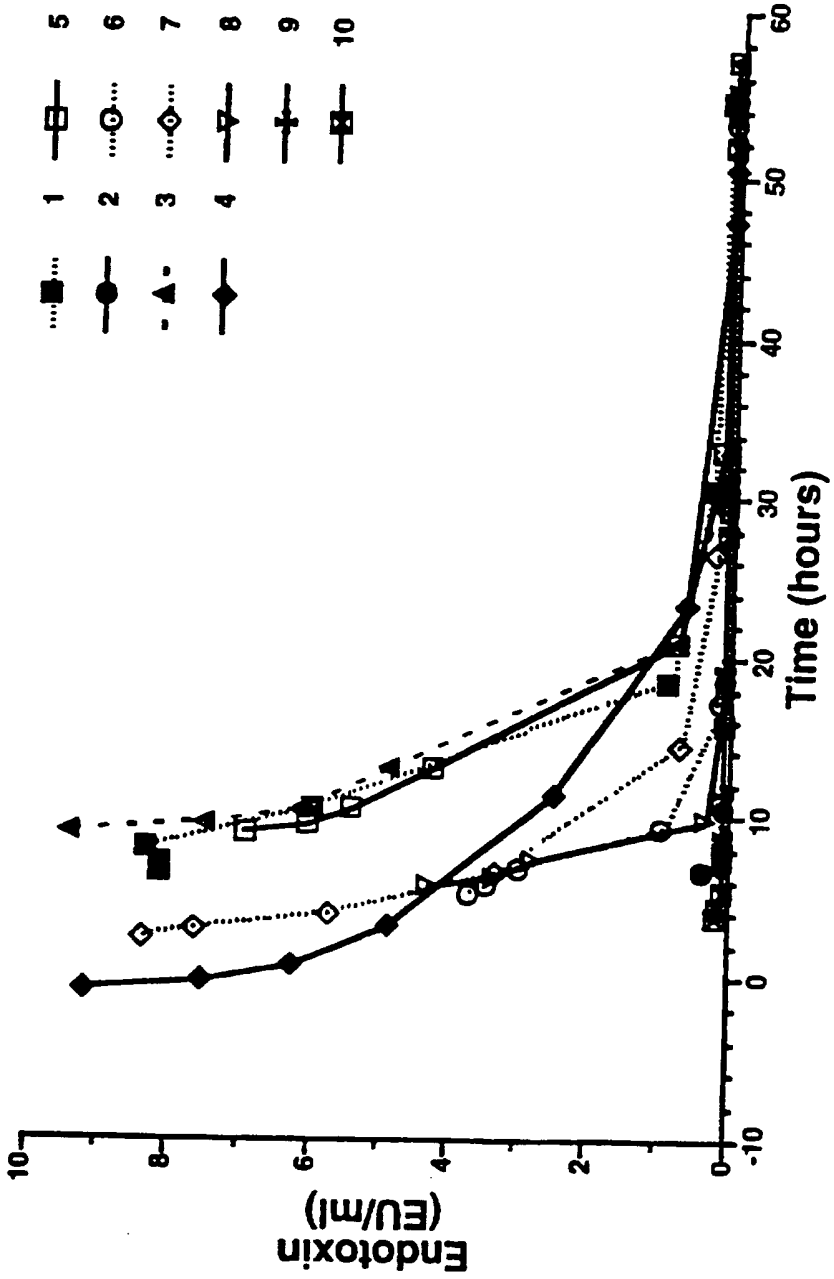
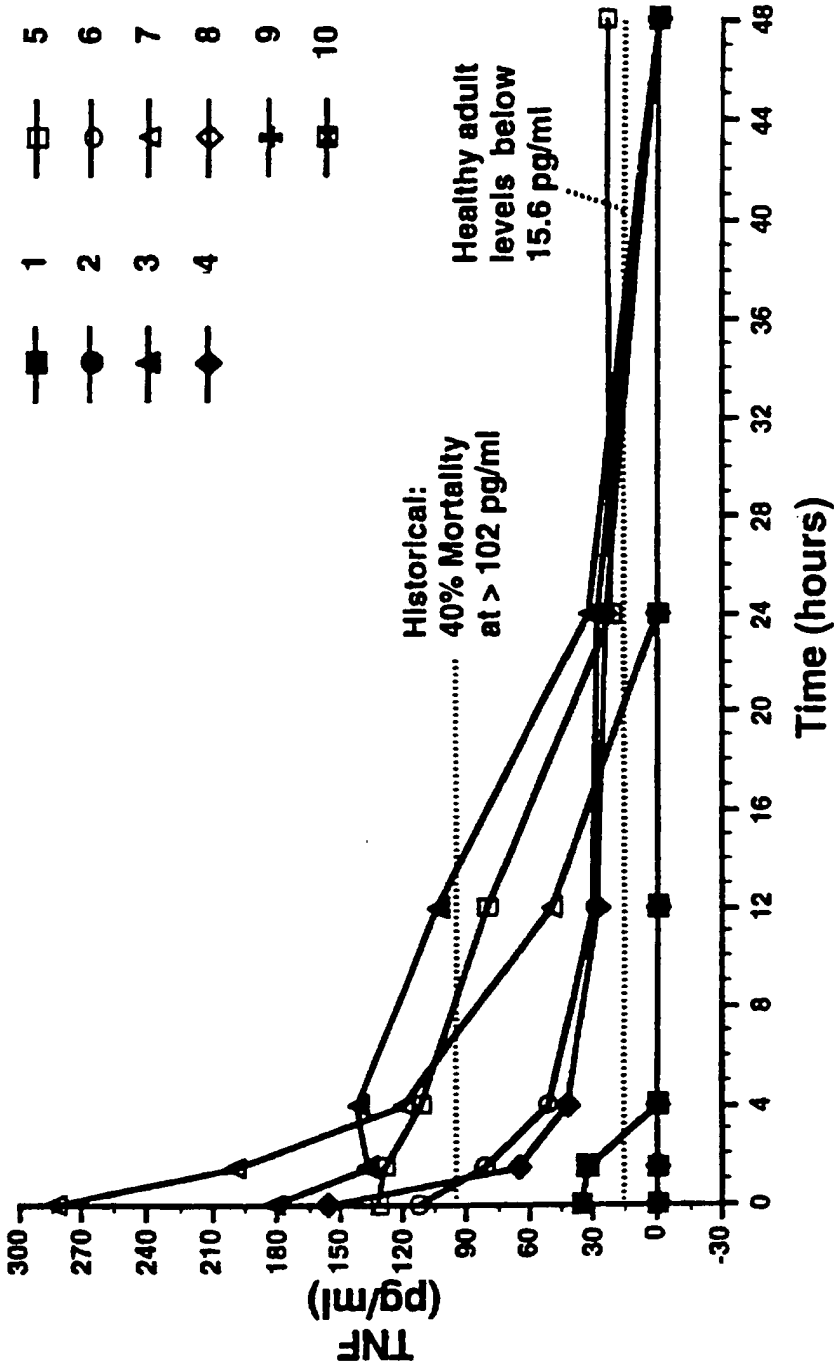

1
2
3
4
5
6
7
8
9
10
Endotoxin (EU/ml)
Time (hours)
10
8
6
4
2
0
-10
0
10
20
30
40
50
60

FIG. 3


TNF (pg/ml)
300
270
240
210
180
150
120
90
60
30
0
-30
0
4
8
12
16
20
24
28
32
36
40
44
48
Time (hours)
Historical: 40% Mortality at > 102 pg/ml
Healthy adult levels below 15.6 pg/ml
1
2
3
4
5
6
7
8
9
10

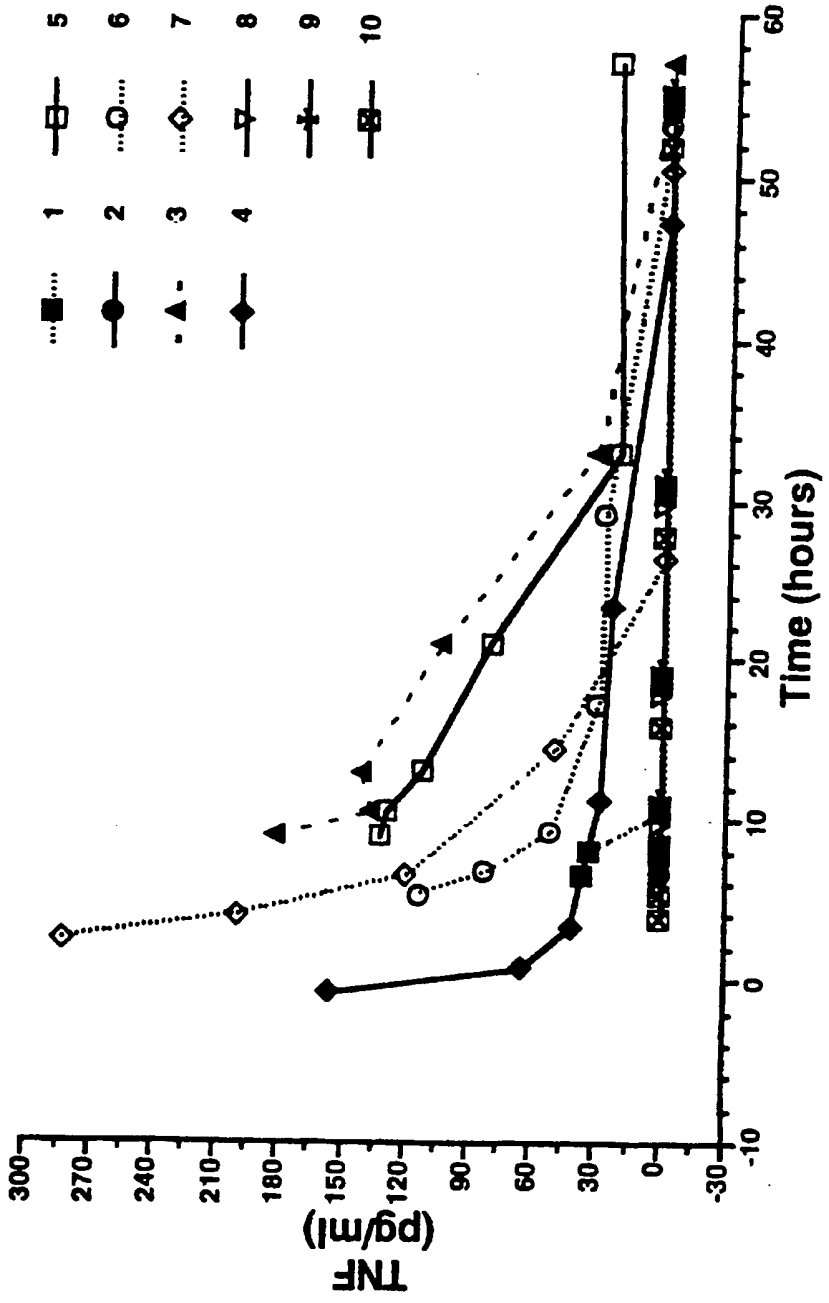
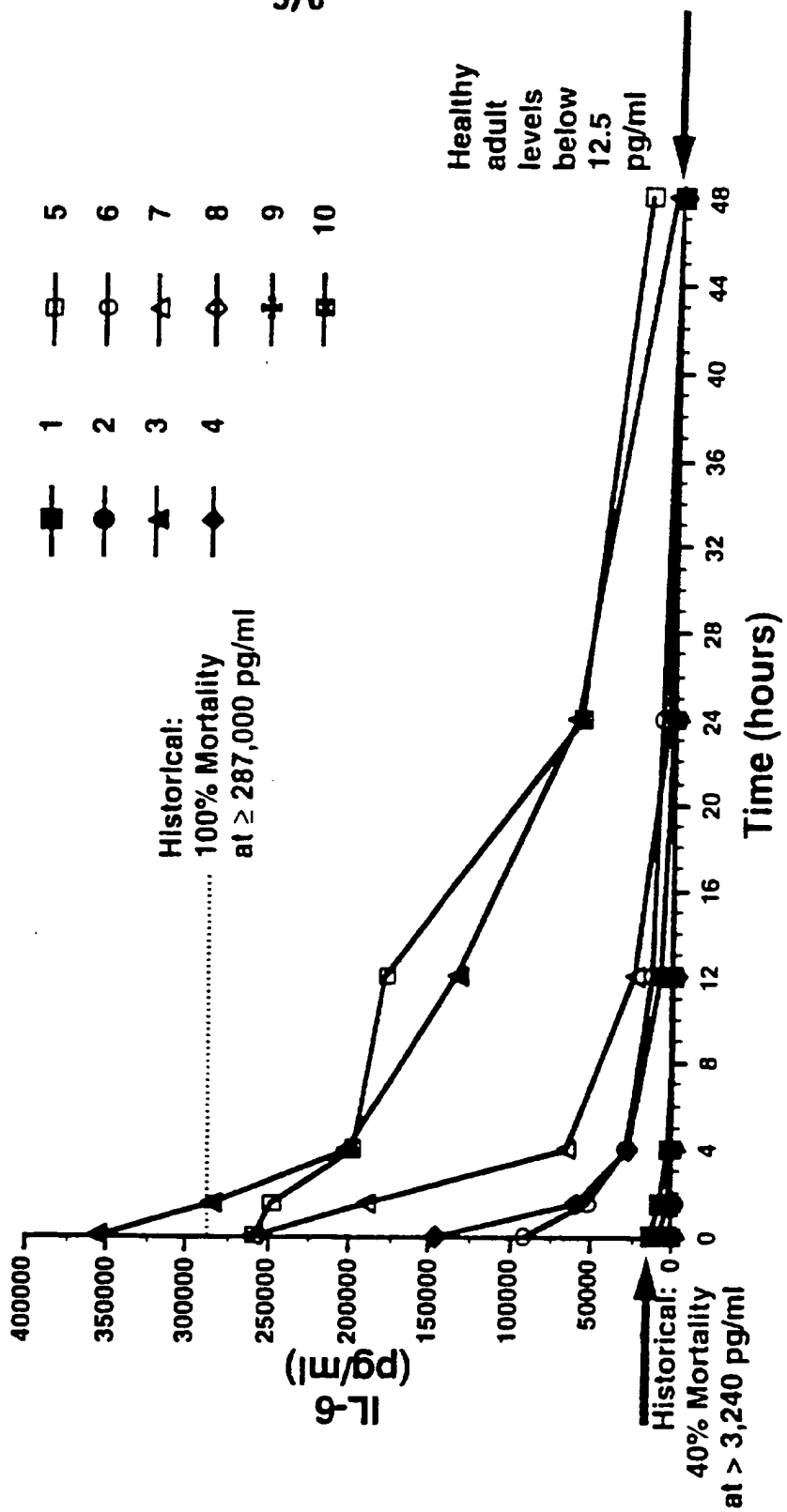

5
6
7
8
9
10
1
2
3
4
TNF (pg/ml)
300
270
240
210
180
150
120
90
60
30
0
-30
-10
0
10
20
30
40
50
60
Time (hours)

FIG. 4

FIG. 5


Healthy adult levels below 12.5 pg/ml
1
2
3
4
5
6
7
8
9
10
Historical: 100% Mortality at ≥ 287,000 pg/ml
IL-6 (pg/ml)
400000
350000
300000
250000
200000
150000
100000
50000
0
Historical: 0 40% Mortality at > 3,240 pg/ml
0
4
8
12
16
20
24
28
32
36
40
44
48
Time (hours)

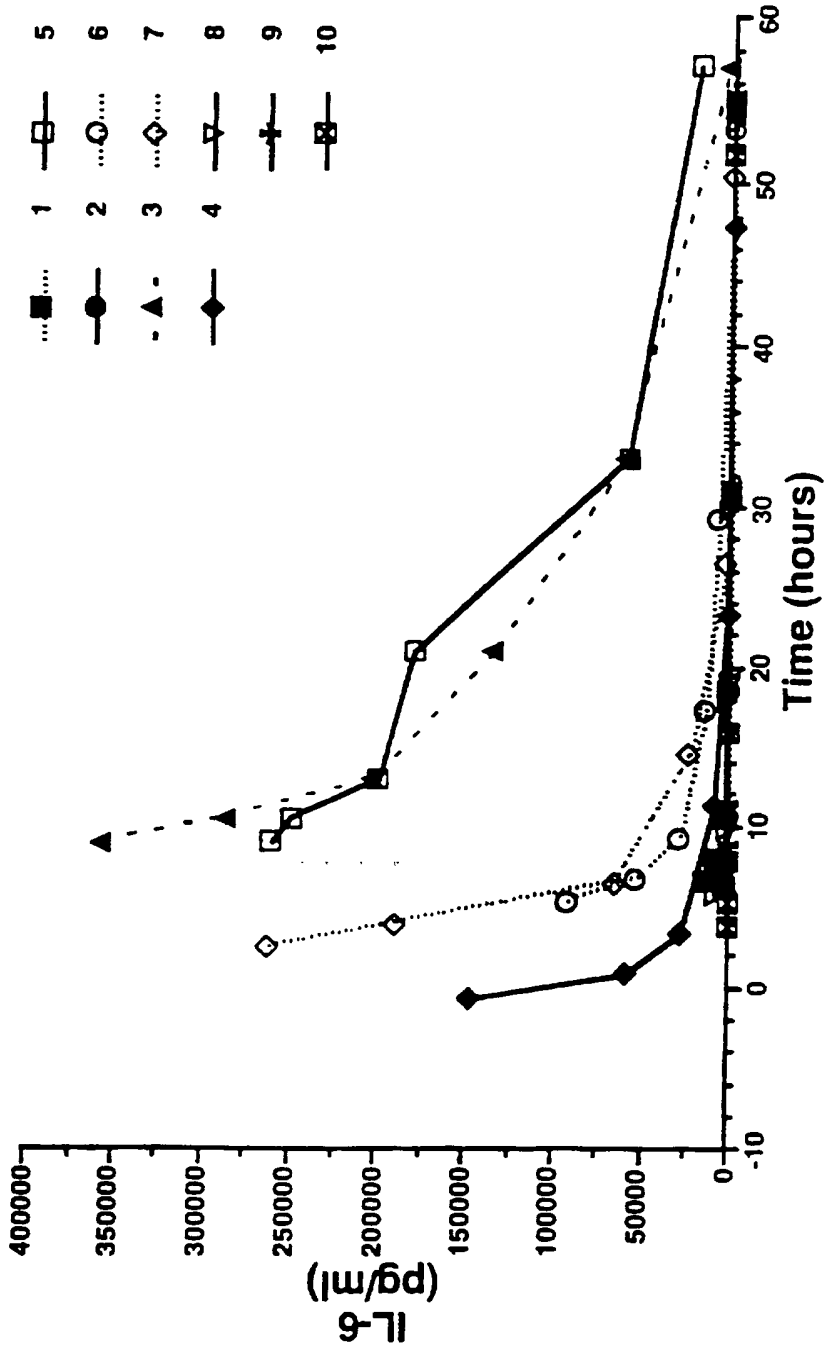

IL-6
(pg/ml)
400000
350000
300000
250000
200000
150000
100000
50000
0
-10
0
10
20
30
40
50
60
Time (hours)
1
2
3
4
5
6
7
8
9
10

FIG. 6

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/08016

A. CLASSIFICATION F SUBJECT MATTER
IPC 6 A61K38/17

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	FASEB JOURNAL FOR EXPERIMENTAL BIOLOGY, vol. 8, no. 4, 15 March 1994, BETHESDA, MD US, page A137 XP002038943 M.B. THORNTON ET AL.: "BACTERICIDAL/PERMEABILITY INCREASING PROTEIN INHIBITS TUMOR NECROSIS FACTOR RELEASE IN WHOLE BLOOD IN RESPONSE TO NEISSERIA MENINGITIDIS AND N. GONORRHOEAE." cited in the application see abstract nr.794 ---	1-7
A	WO 94 25476 A (INCYTE PHARMACEUTICALS, INC.) 10 November 1994 cited in the application see page 45, line 31 - page 46, line 15 --- -/--	1-7

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
26 August 1997	19.09.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Ryckebosch, A

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/08016

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	THE JOURNAL OF INFECTIOUS DISEASES, vol. 171, no. 4, April 1995, CHICAGO, IL, US, pages 948-953, XP002038944 M. KARTALIJA ET AL.: "EFFECT OF A RECOMBINANT N-TERMINAL FRAGMENT OF BACTERICIDAL/PERMEABILITY-INCREASING PROTEIN (rBPI-23) ON CEREBROSPINAL FLUID INFLAMMATION INDUCED BY ENDOTOXIN." see the whole document ---	1-7
A	WO 95 08344 A (XOMA CORPORATION) 30 March 1995 see claims & US 5 523 288 A cited in the application ---	1-7
P,X	PEDIATRIC RESEARCH, vol. 41, no. 4 PART 2, April 1997, BALTIMORE, MD, US, page 35A XP002038945 E.A. KIRCH ET AL.: "PHASE I/II TRIAL OF rBPI-21 (A RECOMBINANT 21kD FRAGMENT OF BACTERICIDAL/PERMEABILITY-INCREASING PROTEIN) IN CHILDREN WITH SEVERE MENINGOCOCCEMIA." see abstract nr.196 -----	1-7

1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 97/08016

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim(s) 1 - 5
is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 97/08016

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9425476 A	10-11-94	AU 6942994 A	21-11-94
		EP 0760849 A	12-03-97
		JP 8511682 T	10-12-96
WO 9508344 A	30-03-95	AU 8074094 A	10-04-95
		CA 2172245 A	30-03-95
		EP 0759774 A	05-03-97
		JP 9502987 T	25-03-97
		US 5523288 A	04-06-96
		ZA 9407394 A	15-05-95